1H-Isoindole-1,3-(2H)-dione, 2-(cyclohexylthio)-
17796-82-6

201-14927B

Molecular Formula: C14-H15-O2-N-S
Molecular Weight: 261.34

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance:	Organic
B. Physical State:	Off-White solid
C. Purity:	95-98% Typical for Commercial Products

1.2 SYNONYMS

Santogard® PVI
Vulkalent® G
Accitard® RE
Duslin® P
N-(Cyclohexylthio)phthalimide
N-Cyclohexylsulfenylphthalimide
PVI
CTP
CTPI

1.3 IMPURITIES

Phthalimide (CAS# 85-41-6) 0.5-2.0%

1.4 ADDITIVES

Butyl Oleate (142-77-8) 0.5-1.5%

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	92.6°C
Decomposition:	No
Sublimation:	No
Method:	FF83.9-1 Initial and Final Melting Point of Organic Compounds, 1996
GLP:	Yes
Remarks:	Instrumental - Capillary Tube Method. Typical range for initial to final melt point determinations is 90-94°C
Reference:	ASTM D-1519 / Flexsys Physical Methods of Analysis
Reliability:	(1) Valid without restriction

*2.2 BOILING POINT

Value:	Decomposition at 196 °C
Pressure:	1013 hPa
Decomposition:	Yes
Method:	MCL 3.9.71, 1971
GLP:	No data
Remarks:	Slow degradation noted at temperatures above 156°C Phthalimide sublimes at decomposition temperature
Reference:	Flexsys Process Manual for Santogard PVI, 1996
Reliability:	(1) Valid without restriction

†2.3 DENSITY

Type:	Density
Value:	1.33
Temperature:	25 °C
Method:	FF97.8-1, Density of Solids by Displacement, 1997
GLP:	Yes
Remarks:	Density of solids by displacement in kerosene
Reference:	FF97.8-1, Flexsys Standard Methods of Analysis, 1997
Reliability:	(1) Valid without restriction

*2.4 VAPOUR PRESSURE

Value:	5.4 E-008 hPa
Temperature:	25 °C
Method:	Calculated – Modified Grain Method MPBPWIN v1.40, 2000
GLP:	No
Remarks:	Calculation based on molecular structure and measured melting Point, Log Kow and water solubility
Reference:	EPIWIN/MPBPWIN v1.40, 2000
Reliability:	(2) Valid with restrictions – modelling data

Value:	<10 E-006 Torr
Temperature:	25 °C
Method:	No data
GLP:	No data
Remarks:	Volatility: No weight loss noted at 170°C
Reference:	Chemical Hazard Information Profile (CHIP) of CTP, 1989
Reliability:	(2) Valid with restrictions – no method detail

*2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow:	3.66
Temperature:	25 °C
Method:	Measured Shake Flask/GC Method for Pow, 1978
GLP:	No
Remarks:	Octanol/water partition coefficient of the test compound was measured by preparing 1% and 0.1% solutions in 100 ml of n-octanol. A 50 ml aliquot of the octanol solution and 500 ml purified water were combined in a 1 liter glass bottle with a foil-lined cap and shaken in the dark for 48 hours. The mixture was allowed to stand quiescent for several days. The aqueous phase was centrifuged for 1 hour at 15,000 rpm and analyzed via HPLC. The n-octanol phase was also analyzed via GC. The partition coefficient, P, was calculated using the equation P = Co/Cw, where Co and Cw are the concentrations of the test compound in octanol and water, respectively. The Pow was 4600 +/- 2200, corresponding to a Log Pow between 3.38 and 3.83.
Reference:	Monsanto ES-78-SS-20, December, 1978
Reliability:	(2) Valid with restrictions – lack of method detail

*2.6 WATER SOLUBILITY

A. Solubility

Value:	22 ppm (mg/L)
Temperature:	25°C
Description:	Of very low solubility
Method:	Saturated Solution / GC Analysis, 1978
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	The aqueous solubility was determined by adding 1g of the test compound and 500ml purified water to a 1 liter glass bottle with an aluminium foil lined cap. The solution was mixed with a magnetic stirrer for several days to produce a saturated solution. Equilibration was performed in the dark to preclude photodegradation. Stirring was stopped one hour before sampling to permit phase (aqueous/solid) separation. The aqueous phase was centrifuged at 15,000 rpm for one hour to remove any suspended particles and then analyzed via GC. Duplicate runs were made after additional stirring to insure a saturated solution. From the GC analysis, it was apparent that the test compound was stable in the air-equilibrated water solution
Reference:	Monsanto ES-78-SS-20, December, 1978
Reliability:	(1) Valid without restriction

Value:	18 mg/L
Temperature:	23°C
Description:	Of very low solubility
Method:	Saturated solution, HPLC analysis, 1978
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	Data from Analytical/Physical Laboratory Report AP78-33341.4
Reference:	Flexsys Process Manual for Santogard PVI, 1996
Reliability:	(1) Valid without restriction

Value:	10.8 mg/L
Temperature:	20 °C
Description:	Of very low solubility
Method:	Saturated Solution / Solvent Extraction / HPLC Analysis, 1986
GLP:	Yes
Remarks:	Preliminary solubility study for Phase I Hydrolysis Study
Reference:	Monsanto ABC 32456, Analytical Bio-Chemistry Laboratories, February, 1986
Reliability:	(1) Valid without restriction

B. pH Value, pKa Value

Not Applicable

2.7 FLASH POINT

Value:	177 °C
Type:	Cleveland Open Cup
Method:	ASTM D 92-96, 1992
Reference:	Flexsys America Data, Test Method for Flash Points and Fire Points by Cleveland Open-Cup Apparatus, ASTM D 92-96
Reliability:	(1) Valid without restrictions

2.11 OXIDISING PROPERTIES

†2.12 OXIDATION: REDUCTION POTENTIAL

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

B. Other data – Henry's Law Constant

Results:	6.40E-008 atm-m3/mole
Remarks:	Calculated at 25°C using molecular structure and measured water solubility of 22 mg/l, measured melting point and Log Kow
Reference:	EPIWIN/HENRYWIN v3.10, 2000
Reliability:	(2) Valid with restrictions – modelling data

3. ENVIRONMENTAL FATE AND PATHWAYS

***3.1.1 PHOTODEGRADATION**

Type:	Air
Indirect Photolysis:	
Type of sensitizer:	OH
Concentration of sensitizer:	156000 molecule/m3
Rate constant (radical):	45.7076E-12cm^3/molecule-sec
Degradation:	50% after 2.808 hours
Method:	Calculated AOP Program v1.90, 2000
GLP:	No
Test substance:	Other (calculated) based on molecular structure and measured water solubility, melting point and Log Kow
Reference:	EPIWIN/AopWin v1.90
Reliability:	(2) Valid with restrictions – accepted calculation method

***3.1.2 STABILITY IN WATER**

Type:	Abiotic (hydrolysis)
Half life:	23.3 hours at pH 7.0 at 25 °C
Degradation:	99.7% at pH 7.0 at 25 °C after 7 days
Method:	Suffet, et al., Hydrolysis Protocols – Effects of Water on the Environmental fate of Chemicals. Test Protocols for Environmental Fate and Movement of Toxicants, Washington, D.C., 1980
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 99+%

Remarks:	Primary stock solutions of 1.00 mg/ml of the test compound were prepared in HPCL-grade Acetonitrile. Subsequent dilutions for spiking and HPLC standards were also prepared in HPLC-grade Acetonitrile. Based on the solubility of the test compound, a sample size of 100 ml was chosen. Test samples were extracted with three 15 ml portions of methylene chloride. The extracts were dried by passing them through a funnel containing anhydrous sodium sulphate, and then analyzed via HPLC. The test sample hydrolized 29% at Day 1 and 99.5% at Day 7. Hydrolysis appeared to occur in a non-linear rate. The primary (99+%) hydrolysis product was isolated and identified by GC/MS, HPLC, IR and NMR as N-(Cyclohexylthio)phthalamide, the carboxylic acid (ring-opened half-acid) derivative of the test compound. By mass-balance analysis, this appeared to be the only hydrolysis product formed at Day 7. It was also established during GC analysis that the hydrolysis product reverted to the parent compound upon heating.
Reference:	Monsanto ABC-32456, Analytical Bio-Chemistry Labs, 1986
Reliability:	(1) Valid without restriction

***3.2 MONITORING DATA (ENVIRONMENTAL)**

Type of Measurement:	Plant discharge
Media:	Water
Results:	The test compound was not detected (detection limit 0.1 parts per billion (ppb) in the Nitro, WV plant discharge of treated water into the Kanawa River.
Remarks:	Aqueous waste streams were treated biologically via an activated sludge system. Treatability studies predicted and plant experience confirmed that the organic components in PVI aqueous wastes are reasonably soft.
Reference:	Monsanto Product Assessment Report 4-2531, July, 1978
Reliability:	(1) Valid without restriction

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION

***3.3.1 TRANSPORT**

Type:	Volatility
Media:	Water
Method:	Calculation from EPIWIN VP/WS 2000
Results:	Volatilization half-life from model river: 1.479E+004 hours Volatilization half-life from model lake: 1.615E+005 hours Volatilization Constant from water: 6.4E-008 atm-m3/mole
Remarks:	Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec. Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec. Calculation based on molecular structure and melt point of 92.6°C, water solubility of 22 mg/L and Log Kow of 3.76
Reference:	EPISUITE/EPIWIN 2000
Reliability:	(2) Valid with restrictions – modelling data

***3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

Media: Air-biota-sediment-soil-water
Method: Fugacity level III
EPIWIN v3.10, 2000
Results:

	Mass Amount (%)	Half-life (hrs)	Emissions (kg/hr)
Air	00.283	5.62	1000
Water	26.8	900	1000
Soil	70.7	900	1000
Sediment	2.2	3.6E+003	0

Persistence time estimated at 714 hours
Remarks: Calculations based on molecular structure and measured input values of water solubility 22 mg/L, melting point of 92.6°C and Log Kow of 3.76
Reference: EPISUITE/EPIWIN v3.10, 2000
Reliability: (2) Valid with restrictions – modelling data

***3.5 BIODEGRADATION**

Type: Aerobic
Inoculum: Adapted
Concentration of the chemical: 3 mg/l test substance
Medium: Sewage treatment
Degradation: 33% after 24 hours
47% after 72 hours
59% after 96 hours
77% after 168 hours
99+% after 190 hours
Results: Readily biodegradable
Method: Semi-Continuous Activated Sludge (Primary Biodegradation)
Thompson-Duthie-Sturm Procedure
Monsanto Shake Flask Procedure, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 95%
Remarks: Analytical monitoring involved extraction with methylene chloride, sample concentration, and analysis via a gas chromatograph equipped with dual FID. The test compound showed significant primary degradation. Moderate inhibition of the normal sludge growth was observed during the SCAS test.
Ultimate Biodegradation by Shake Flask Test: 3.4% of theory CO2 evolution after 32 days, indicating that a more persistent metabolite/degradation product is formed during primary biodegradation.
Reference: Monsanto ES-78-SS-28, Environmental Sciences Labs, 1978
Reliability: (1) Valid without restrictions

3.6 BOD5, COD or BOD5/COD Ratio

3.7 BIOACCUMULATION

Species: Other
BCF: 130

Method:	Calculation, Neely, et. al., 1974
GLP:	No data
Remarks:	Calculation from measured octanol/water partition coefficient Pow = 4600 +/- 2200
Reference:	Monsanto ES-78-SS-20, Environmental Sciences Labs, 1978
Reliability:	(2) Valid with restrictions – acceptable calculation method

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test:	static Closed system
Species:	Lepomis macrochirus (Bluegill Sunfish)
Exposure period:	96 hours
Concentrations:	0, 0.49, 0.65, 1.0, 1.4, 1.8 and 2.4 mg/l
Results:	LC_{50} (24h) = 1.5 mg/l LC_{50} (48h) = 1.2 mg/l LC_{50} (96h) = 1.2 mg/l NOEC = 0.49 mg/l LOEC = 0.65 mg/l
Analytical monitoring:	No
Method:	EPA-660/3-75-009 Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972).
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 96.5%.
Remarks:	The test material, in reagent-grade acetone, was introduced into 15 liters of diluent water in all-glass vessels. Test concentrations ranged from 0.49 to 2.4 mg/l for the test compound. The experiment included a control and a solvent (acetone) control. Ten bluegill, standard length 3.8 cm, were added to each test vessel. The test fish were not fed for 48 hours prior to testing, nor during the exposure period. No aeration was provided during the test. Temperature was maintained at 22°C. Dissolved oxygen content ranged from 8.3 mg/l (78% of saturation) at the beginning of the test, to 0.4 mg/l (4% of saturation) at the end of the exposure period. Beginning pH was 7.3; ending pH was 6.8. Water hardness (CaCO3) was 255 ppm. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50 values and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-252, EG&G Bionomics, 1976
Reliability:	(1) Valid without restriction

Type of test:	Static Closed-system
Species:	Oncorhynchus mykiss (Rainbow Trout)
Exposure period:	96 hours
Concentrations:	0, 0.32, 0.42, 0.56, 0.75 and 1.8 mg/l
Results:	LC_{50} (24h) = 0.47 mg/l

	LC_{50} (48h) = 0.42 mg/l LC_{50} (96h) = 0.41 mg/l NOEC = <0.32 mg/l LOEC = 0.32 mg/l
Analytical monitoring:	No
Method:	EPA-660/3-75-009 Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972)
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 96.5%.
Remarks:	The test material, in reagent-grade acetone, was introduced into 15 liters of diluent water in all-glass vessels. Test concentrations ranged from 0.32 to 1.8 mg/l for the test compound. The experiment included both a control and a solvent (acetone) control. Ten rainbow trout, standard length 2.7 cm, were added to each test vessel. The test fish were not fed for 48 hours prior to testing, nor during the exposure period. No aeration was provided during the test. Temperature was maintained at 12°C. Dissolved oxygen content ranged from 9.8 mg/l (92% of saturation) at the beginning of the test, to 5.2 mg/l (49% of saturation) at the end of the exposure period. Beginning pH was 7.6; ending pH was 7.2. Water hardness (CaCO3) was 255 ppm. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50 values and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-252, EG&G Bionomics, 1976
Reliability:	(1) Valid without restriction

Type of test:	Flow-through (dynamic) Open system
Species:	Pimephales promelas (Fathead Minnows)
Exposure period:	14 days (336 hours)
Concentrations:	0, 0.024, 0.038, 0.11, 0.26 and 0.59 mg/l
Results:	LC_{50} (24h) = 0.43 mg/l LC_{50} (192h) = 0.42 mg/l LC_{50} (240h) = 0.40 mg/l LC_{50} (336h) = 0.39 mg/l
Analytical monitoring:	Yes
Method:	EPA-660/3-75-009 Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 99+%
Remarks:	The test fish, mean standard weight 0.56 g and mean standard length 29.3 mm, were obtained from Fattig's Fish Hatchery in Brady, Nebraska. The fish were held in culture tanks on a 16-hour photoperiod and were observed for at least 14 days prior to testing. During the holding, acclimation and test periods, the fish were fed a standard commercial fish food daily in an amount equivalent to 3% of body weight. As a quality check, the fish were challenged with a reference compound, Antimycin A, prior to the test. The observed 96 hr LC50 and 95% confidence limits

indicated that the fish were in good condition. A proportional diluter system was used for the intermittent introduction of the test article, in nanograde acetone, and diluent water, into the test aquaria. Aerated well water, hardness 250 mg/l and alkalinity 360 mg/l, pH 7.7 and dissolved oxygen 9.3 mg/l, was delivered to the glass aquaria at the rate of 300ml/minute, an amount which provided replacement of the 30 liter volume at least 14 times in each 24-hour period. The temperature in the test aquaria was held at 22°C. Water quality parameters of temperature, dissolved oxygen (100-60%), pH (7.8-7.9) and ammonia (0.28-2.0) were monitored throughout the test and remained within acceptable limits. Thirty test fish/aquaria were exposed to concentrations of 0, 0.024, 0.038, 0.11, 0.26 and 0.59 mg/l of the test article for the 14-day test period. Observations for mortality and abnormal behavior were performed once/day. Concentrations of the test article were determined by IR spectroscopy using a calibration curve determined from known concentrations with the addition of Rhodamine B dye. The concentrations were further confirmed by gas chromatography. The statistical methods described by Litchfield and Wilcoxon were used to determine the LC50 values and the 95% confidence limits. From the acute toxicity curves using both the nominal and mean measured water concentrations, it was determined that the lethal threshold had not been reached after 14 days. The results also indicated that the test article did not appear to have cumulative toxicity.

Reference: Monsanto AB78-122B, Analytical Bio-Chemistry Labs, 1979

Reliability: (1) Valid without restriction

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static
Closed system

Species: Daphnia magna

Exposure period: 48 Hours

Concentrations: 0, 10, 18, 32, 56 and 100 mg/l

Results: $EC_{50\ (24h)}$ = 32 mg/l
$EC_{50\ (48h)}$ = 32 mg/l
NOEC = <10 mg/l

Analytical monitoring: No

Method: EPA-660/3-75-009 Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 96.5%

Remarks: The Daphnia magna used in the test were cultured at the ABC facilities. Adult Daphnia were fed an algae and trout chow mixture daily until 24 hours prior to testing. The bioassay was conducted in 500 ml glass beakers containing 250 ml of ABC well water. During the test, dissolved oxygen concentration ranged from 9.1-8.7 mg/l, pH range was 7.7-8.4, hardness (CaCO3) was 250 mg/l, and alkalinity was >250 mg/l. Vessels were kept in a water bath at 19°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial

range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control (0.01ml). Concentrations (in duplicate) of the test substance were 0, 10, 18, 32 or 56 mg/l. Ten daphnia, first instar less than 18 hours old, were placed in each test chamber. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. Statistical analysis of the concentration vs. effect data was calculated employing the techniques of Litchfield and Wilcoxon (1949).

Reference: Monsanto AB-78-122, Analytical Bio-Chemistry Labs, 1978

Reliability: (1) Valid without restriction

***B. Midge**

Type of test:	Static Closed system
Species:	Paratanytarsus parthenogenetica (Midge)
Exposure period:	48 Hours
Concentrations:	0, 18, 32, 56, 100, 180 and 320 mg/l
Results:	LC_{50} (48h) = 130 mg/l NOEC = 32 mg/l LOEC = 56 mg/l
Analytical monitoring:	No
Method:	EPA-660/3-75-009 Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) and Gettings and Adams, Method for Conducting Acute Toxicity Tests with Midge 1980
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	The test midge for this study were cultured at the ABC facilities. The adult midge were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing. The test was carried out using 3rd and 4th instar larvae, 8-10 days old. The static bioassay was conducted in 250 ml glass beakers containing 200 ml of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 8.9 mg/l, hardness (CaCO3) of 255 ppm and pH 7.9. The test vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range finding experiment preceded the definitive bioassay. Nanograde Acetone was used to prepare the test solutions of 18, 32, 56, 100, 180 or 320 mg/l, and as the solvent control. Well water was used as the control. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen content ranged from 8.9 to 7.9 mg/l and pH ranged from 7.9 to 8.3 during the testing. Water quality parameters of temperature, dissolved oxygen content and pH were measured at the termination of the test and were within acceptable limits. The LC50 values were calculated via a computerized program performing the following statistical tests: binomial, moving average and probit tests.

Reference:	Monsanto 9AB981016, Analytical Bio-Chemistry Labs, 1981
Reliability:	(1) Valid without restriction

***4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae**

Species:	Selenastrum capricornutum (Freshwater alga)
Endpoint:	Biomass and Growth rate
Exposure period:	96 Hours
Concentrations:	0, 6, 10, 32, 56 and 100 ppm
Results:	$EC_{50\ (96h)}$ = 22 ppm for a chlorophyll, 21 ppm for cell numbers NOEC = ~6 ppm LOEC = Not Determined
Analytical monitoring:	No
Method:	US EPA Algal Test Procedure: Bottle Test, 1971 Closed system
GLP:	No data
Results:	The test algae were obtained from the US EPA Environmental Research Laboratory in Corvallis, Oregon. Beginning cell numbers in the test flasks were 2.0 x 10(4) cells/ml. Cultures were incubated at 24°C under approximately 4,000 lux illumination. Triplicate cultures were employed for each of the test concentrations and the control. Test containers were 125ml flasks containing 50ml of test medium. Concentrations for the definitive test were based on the results of a 96-hr range-finding study. These concentrations were 0, 6, 10, 32, 56 or 100 ppm, plus a solvent control (acetone, 0.05 ml). The measured pH values ranged from 7.8 to 7.0 during the course of the testing. There were no other water quality measurements besides temperature and pH reported in this study. Statistical analysis involved converting each test concentration to a logarithm, and the corresponding percentage decrease of in vivo chlorophyll a or cell numbers was converted to a probit (Finny, 1971). The EC50s and 95% confidence limits were then calculated by linear regression. The toxicity of the test substance to algae was similar throughout the 96 hours of exposure. There was no significant difference between growth of the control and solvent control cultures after 96 hours of exposure by either measured parameter.
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	Both a chlorophyll and cell numbers measured to confirm results.
Reference:	Monsanto BN-78-1384317, EG&G Bionomics, 1978
Reliability:	(2) Valid with restrictions – no GLP statement

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_{50}
Species/strain:	Rats, Sprague-Dawley Albino
Value:	2600 mg/kg bw
Sex:	Male and female
# of Animals:	20
Vehicle:	Corn Oil, 20% suspension

Doses: 1580, 2000, 2510 or 3160 mg/kg bw
Recovery Period: 14 days
Method: Single Oral Dose, Younger Laboratories Protocol, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: Four groups of male and female rats (5/sex/dose level) were fed a single oral dose of the test article as a 20% suspension in corn oil via oral gavage. Male rats had initial body weights of 220-250 grams: females had initial body weights of 215-230 grams. Clinical signs of toxicity included reduced activity and appetite for 2-3 days for survivors, and increasing weakness, collapse and death for decedents in 1-3 days. Gross autopsy findings on decedents were hemorrhagic areas of the lungs, liver discoloration, and gastrointestinal inflammation. Survivors were sacrificed after a 14-day observation period. All viscera appeared normal in these animals. Statistical calculation of the LD50 was done according to the method of de Beer. The 95% confidence limits were 7380-9100 mg/kg.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
1580	0/2	0/3	0/5
2000	0/3	0/2	0/5
2510	1/2	1/3	2/5
3160	2/3	2/2	4/5

Reference: Monsanto Y-73-259, Younger Laboratories, 1974
Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: LD_{50}
Species/strain: Rats, Sprague-Dawley Albino
Value: 8200 mg/kg bw
Sex: Male and female
# of Animals: 20
Vehicle: Corn Oil, 25% suspension
Doses: 5010, 6310, 7940 or 10,000 mg/kg bw
Recovery Period: 14 days
Method: Single Oral Dose, Younger Laboratories Protocol, 1976
GLP: No data
Test substance: Other TS: Santogard PVI 25, Purity: 25% (on inert carrier)
Remarks: Four groups of male and female rats (5/sex/dose level) were fed a single oral dose of the test article as a 25% suspension in corn oil via oral gavage. Male rats had initial body weights of 225-250 grams: females had initial body weights of 220-235 grams. Clinical signs of toxicity included reduced activity and appetite for 2-3 days for survivors, and increasing weakness, tremors, collapse and death for decedents in 1-3 days. Gross autopsy findings on decedents were hemorrhagic areas of the lungs, liver discoloration, and gastrointestinal inflammation. Survivors were sacrificed after a 14-day observation period. All viscera appeared normal in these animals. Statistical calculation of the LD50 was done according to the method of de Beer. Reported 95% Confidence Limits: 7380-9100 mg/kg bw.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
5010	0/3	0/2	0/5
6310	1/2	1/3	2/5
7940	2/3	0/2	2/5
10,000	2/2	2/3	4/5

Reference: Monsanto Y-76-4, Younger Laboratories, 1976
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_{50}
Species/strain: Rabbits, New Zealand Albino
Value: >5010 mg/kg bw
Sex: Male/female
# of Animals: Three
Vehicle: Corn Oil
Doses: 5010 or 7940 mg/kg bw
Exposure Time: 24 Hours
Recovery Period: 14 days
Method: Single Dermal Dose, Younger Laboratories Protocol, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96 %
Remarks: The test substance, as a 40.0% suspension in corn oil, was applied to the shaved skin of male and female rabbits for 24 hours as single dermal application at dose levels of 5010 or 7940 mg/kg/body weight. The male weighed 2.0 kg, and the females from 1.8-1.9 kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical observations were made three times during the first eight hours after dosing, and twice daily thereafter until sacrifice on Day 14. Clinical signs of toxicity included reduced appetite and activity (two to four days in survivors), followed by increasing weakness, collapse and death (Day 4). Findings from the gross autopsy on decedents indicated hemorrhagic areas of the lungs, slight liver discoloration, and gastrointestinal inflammation. Gross autopsy reports on survivors indicated that all viscera appeared normal.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
5010	0/1	---	0/1
7940	0/1	1/1	½

Reference: Monsanto Y-73-259 Younger Laboratories, 1974
Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: LD_{50}
Species/strain: Rabbits, New Zealand Albino
Value: >7940 mg/kg bw

Sex:	Male/female
# of Animals:	Three
Vehicle:	Corn Oil
Doses:	5010 or 7940 mg/kg bw
Exposure Time:	24 Hours
Recovery Period:	14 days
Method:	Single Dermal Dose, Younger Laboratories Protocol, 1976
GLP:	No data
Test substance:	Other TS: Santogard PVI 25, Purity: 25% (on inert carrier)
Remarks:	The test substance, as a 40.0% suspension in corn oil, was applied to the shaved skin of male and female rabbits for 24 hours as single dermal application at dose levels of 5010 or 7940 mg/kg/body weight. The male weighed 1.8 kg, and the females from 1.8-2.0 kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical observations were made three times during the first eight hours after dosing, and twice daily thereafter until sacrifice on Day 14. Clinical signs of toxicity included reduced appetite and activity (one to three days). All animals survived until terminal sacrifice. All viscera appeared normal in these animals.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
5010	---	0/1	0/1
7940	0/1	0/1	0/2

Reference:	Monsanto Y-76-4 Younger Laboratories, 1976
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.2.1 SKIN IRRITATION/CORROSION

Species/Strain:	Rabbits, New Zealand Albino
Sex:	Male and female
# of Animals:	6 in each study, 30 total
Vehicle:	None
Value:	0.8/8.0 [Y-79-54, Lot # NL-02-041] 0.1/8.0 [Y-79-55, Lot # NL-02-003] 0.2/8.0 [Y-79-56, Lot # NL-01-043] 0.4/8.0 [Y-79-57, Lot # NK-05-046] 0.6/8.0 [Y-79-58, Lot # NL-02-027]
Results:	Non-irritating
Classification:	Not Irritating
Method:	F.H.S.A. and Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 96.5 – 97.1%
Remarks:	0.5 grams of the test substance (from five different lots manufactured over a two-month period) as a finely ground powder moistened with water, was applied to the shaved dorsal areas of six albino rabbits. The test material was applied to the skin under 1" square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and gauze patches

	were removed after 24 hours. Dermal irritation was scored by the Draize Method, and results were recorded 24, 48, 72 and 168 hours after topical application. The Primary Irritation Index was calculated by averaging the mean scores at 24 and 72 hours. The Primary Irritation Index ranged from 0.1 to 0.8 on a scale of 0.0-8.0.
Reference:	Monsanto Y-79-54, Younger Laboratories, May, 1979 Monsanto Y-79-55, Younger Laboratories, May, 1979 Monsanto Y-79-56, Younger Laboratories, May, 1979 Monsanto Y-79-57, Younger Laboratories, May, 1979 Monsanto Y-79-58, Younger Laboratories, May, 1979
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.2.1 EYE IRRITATION/CORROSION

Species/Strain:	Rabbits, New Zealand Albino
Sex:	Male and female
# of Animals:	Six in each study, 30 total
Vehicle:	None
Value:	24.6/110.0 [Y-79-54, Lot # NL-02-041] 23.0/110.0 [Y-79-55, Lot # NL-02-003] 23.5/110.0 [Y-79-56, Lot # NL-01-043] 23.5/110.0 [Y-79-57, Lot # NK-05-046] 22.3/110.0 [Y-79-58, Lot # NL-02-027]
Results:	Mildly irritating
Classification:	Eye Irritant
Method:	F.H.S.A. and Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP:	No data
Test substance:	As prescribed in 1.1-1.4, purity: 96.5 – 97.1%
Remarks:	70-80 mg of the test substance (from five different lots manufactured over a two-month period) as a finely ground powder was applied to one eye of six albino rabbits. The other eye was not treated and served as a control. The cornea, iris and conjuntivea were examined immediately after treatment, and then at intervals of 10 minutes, 1 hour, and at 24, 48, 72 and 168 hours, and then again at 10 and 21 days. The Draize Method was used for scoring eye irritation. Findings: Immediate: Moderate discomfort, eyes tightly closed 10 minutes: Moderate erythema, very slight edema, copious discharge 1 hour: Iris reaction to light sluggish, severe erythema, slight to moderate edema, copious discharge 24 hours: Areas of barely perceptible corneal dullness, iris reaction to light sluggish, severe erythema (necrosis), slight edema, copious discharge containing much whitish discharge (with blood) 48 - 168 hours: Gradual improvement. Very slight ulceration in some animals 10 days: Slight ulceration in a few animals 21 days: Slight ulceration in 1-2 animals; other scored "0" The average Draize score for 24, 48 and 72 hours was calculated for each animal and then averaged over the six animals. The Draize scores ranged from 22.3-24.6 on a scale from 0-110.0
Reference:	Monsanto Y-79-54, Younger Laboratories, May, 1979

Monsanto Y-79-55, Younger Laboratories, May, 1979
Monsanto Y-79-56, Younger Laboratories, May, 1979
Monsanto Y-79-57, Younger Laboratories, May, 1979
Monsanto Y-79-58, Younger Laboratories, May, 1979

Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.3 SKIN SENSITISATION

Type: Skin Patch Test
Species/strain: Human
# of subjects: 37 and 30 (two test panels), 67 total
Result: 15/37 positive reactions
10/30 positive reactions
Sensitizing; also primary and cumulative irritant
Method: Shelanski Repeated Insult Patch Test, 1953
Test substance: As prescribed in 1.1-1.4, purity: 96.5%
Remarks: The test material was applied to an adhesive patch so as to cover approximately 1 square inch. The patch was applied to the upper arm or back of 55 human volunteers. After 24 hours, the patch was removed and the application site examined for reactions. After a 24-hour rest period, the patch was applied as before. When eight such applications had been completed, a two week rest period was allowed, after which a challenge application was made in the same manner as before. All applications were made to the same site. Evidence of primary irritation was observed in 10 of the 67 subjects following the initial application. During the subsequent serial applications, severe irritation was observed in 43 of the 67 subjects. Under the conditions of this test, Santogard PVI was considered to be a primary and cumulative skin irritant, as well as a sensitizer.
Reference: Monsanto SH-66-10, Industrial Biology Laboratories, 1966
Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: Skin Patch Test
Species/strain: Human
# of subjects: 53
Result: Not sensitizing; mild cumulative irritation
Method: Shelanski Repeated Insult Patch Test, 1953
Test substance: Other TS: Compounded, uncured rubber stock containing 2 pounds PVI per 100 pounds rubber (2 phr)
Remarks: Patches 1" square of the rubber stock were applied to the skin of 53 human volunteers and left in place for 24 hours. Readings were taken at the end of 24 hours, and the sites were rested for an additional 24 hours. Patches were reapplied and read again after 24 hours. Fifteen such applications were used. A 10-day rest period followed, and then the challenge application was applied for 24 hours. No positive reactions were observed following the initial application, any subsequent application or after the challenge application. Only mild cumulative irritation was observed in this study. The level used in this study, 2 parts per hundred rubber (2 phr) is about five times higher than what is normally used in compounded rubber stocks.
Reference: Monsanto SH-69-11, Industrial Biology Laboratories, 1970

Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: Skin Patch Test
Species/strain: Human
# of subjects: 310
Result: Sensitizing
Method: No data
Test substance: As prescribed by 1.1-1.4; purity: 'commercial'
Remarks: In a survey of 310 subjects patch tested with a series of thirty different rubber additives over a four-year period, 11 subjects (3.5%) showed an allergic reaction to CTP, N-(cyclohexylthio)phthalimide. Four of the CTP-sensitive patients reacted only to CTP, while the other seven reacted to other rubber chemicals as well. The authors concluded that CTP does no appear to cross-react with other rubber chemicals.
Reference: Kanerva et al., Contact Dermatitis 34, 1996
Reliability: (4) Not classifiable – data from a secondary literature source

*5.4 REPEATED DOSE TOXICITY

Species/strain: Rats, Sprague-Dawley CD
Sex: Male/Female
# of animals: 60 (30 male, 30 female)
Route of Administration: Dietary
Exposure period: 4 weeks
Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 50, 150, 300, 600 or 1500 ppm
Control group: Yes; Concurrent vehicle
NOEL: 300 ppm
LOEL: 600 ppm (based on body weight reduction)
Results: In a 30-day range-finding study that preceded a 24 month study, the test substance was administered orally, via dietary admixture, to groups of six-week old CD male and female rats (5/sex/group). Control animals received the standard laboratory diet. Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at study termination. There were no mortalities during the course of the study. After four weeks of treatment, all animals were sacrificed, selected organs were weighed, and organ/body weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Statistical evaluations included mean body weight, mean food consumption, mean clinical laboratory values, and mean terminal organ/body weight and organ/body weight ratios via the appropriate one-way analysis of variance technique, followed by a multiple comparison procedure. Calculations for the statistical significance of differences were performed according to the method of Dunnett (1955). Differences from control in mean body weights were statistically significant at 600 ppm and 1500 for both males and females. The reduced body weights were the only treatment-related effects observed. The stability of the test article

on rat feed, the HPLC analytical method of analysis, and the rat feed/test article homogeneity/mixing efficiency were also validated during this study.

Method: Other: PRL Protocol, 1977, approved by study sponsor
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.2%
Reference: Monsanto PR-78-109A, Pharmacopathics Research Laboratories, 1978
Reliability: (1) Valid without restriction

Species/strain: Rats, Charles River Albino (COBS)
Sex: Male/Female
# of animals: 40
Route of Administration: Inhalation
Exposure period: 28 days
Frequency of treatment: 6 hr/day, 5 days/week for 4 weeks. (Total = 20 exposures)
Post exposure observation period:
Dose: 0, 52, 157 or 536 mg/m3
Control group: Yes; Concurrent no treatment
NOEL: >536 mg/m3
LOEL: Not determined
Results: Four groups of 5 male and 5 female young adult albino rats were exposed to either zero, low, intermediate or high dust concentrations of the test article. Test dusts were suspended in streams of clean, dry air, and introduced through the top center of exposure chambers and exhausted out the bottom. HPLC analytical testing confirmed concentrations and total weight of test dusts. Observations were made with respect to incidence of mortality, reactions displayed and body weight effects. Hematologic and clinical chemistry studies and urinalyses were conducted on all test and control animals on Day 23. All animals survived until sacrifice on Day 28. A complete set of organs and tissues was removed from each animal and preserved in formalin. Histopathologic studies were conducted on selected tissues and organs from the control and high concentration groups. Weights of selected organs were recorded and subjected to statistical analyses. A sample of the airborne dust was collected weekly from the test atmosphere for particle size determination. Statistical calculations were performed via computerized programs that utilized Scheffe's Multiple Comparison Test, Tukey's Multiple Comparison Test and analysis of variance. Findings: No adverse effects observed. No significant differences were noted in the weights of the brains, gonads and hearts of treated animals when compared to controls. No gross or histopathologic alterations attributed to the test article were observed in any of the treated animals.
Method: Other: IBT Subacute Dust Inhalation Protocol (Audited), 1976
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.2%
Reference: Monsanto BTL-76-193 Industrial Bio-Test Laboratories, 1977
Reliability: (1) Valid without restriction

Species/strain: Rats, Sprague-Dawley CD
Sex: Male/Female
# of animals: 120 (15/sex/dose)
Route of Administration: Inhalation
Exposure period: 90 days (13 weeks)
Frequency of treatment: 6 hr/day, 5 days/week
Post exposure observation period:
Dose: 0, 15, 50 or 150 mg/m3
Control group: Yes; Concurrent no treatment
NOEL: 15 mg/m3 for females
Not determined for males
LOEL: Not determined
Results: Exposure levels were monitored gravimetrically five times per chamber per day. Four samples per chamber per week were collected in impingers and analyzed via HPLC. Particle size analysis indicated that >90% of the test atmosphere was respirable to the rat. Young adult rats, age 7 weeks, wee used for this study. The average weight range for males was 160-250 grams, and 130-180 grams for females. Observations of test animals were made twice daily. Detailed physical examinations were conducted once per week. Body weights were recorded pre-test, weekly during the study, and prior to sacrifice. Blood specimens for haematology and clinical chemistry evaluations were collected from 20 pre-test animals (10/sex) and from 10 animals/sex/dose after the sixty-fifth exposure. Complete gross post-mortem examinations were conducted on all animals. Histopathological evaluations were performed on selected tissues from all animals from the control and high-dose groups. The lungs, kidneys and tissues and organs with gross lesions and masses from all animals were examined microscopically.

Statistical analysis: Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure as needed. (Bartlett's test, standard one way ANOVA using the F distribution, Dunnett's test, Kruskal-Wallis test).

Findings: All animals, with the exception of one mid-dose male, survived to terminal sacrifice. This animal's death was considered unrelated to treatment. Excess lacrimation, mucoid nasal discharge, and dried red nasal discharge/red material around the facial area were exhibited by most treated animals of both sexes throughout the study. A few mid- and high-dose males and females exhibited rales throughout most of the study. High-dose males and females, along with mid-dose females, exhibited decreased body weights. Elevations in kidney weight were found in high-dose males. Males showed dose-related increases in incidence of kidney lesions which were characterized by eosinophilic droplets in the proximal tubule, degeneration and regeneration of the tubular epithelium, and granular casts occluding and causing dilation of renal tubules. Scattered granulomas of the lung were noted in controls and treated animals, but more frequently in the high-dose males. Discoloration of the kidneys, primarily pallor, was observed grossly in a number of treated males, but most frequently in the high-dose males. Ophthalmological results and clinical chemistry

results for treated animals were evaluated as unremarkable. The no-effect level was not established for males in this study, as toxicologically significant, treatment-related effects were exhibited by males at all dose levels. The no-effect level for females was established at 15 mg/m3.

Method: Other: Bio/dynamics Protocol – approved by study sponsor, 1984
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 98%
Reference: Monsanto BO-84-162, Bio/dynamics, Inc., 1986
Reliability: (1) Valid without restriction

Species/strain: Rats, Sprague-Dawley CD
Sex: Male/Female
# of animals: 600 (75/sex/dose)
Route of Administration: Dietary
Exposure period: 23 months for male rats
24 months for female rats
Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 50, 150 or 500 mg/kg/day
Control group: Yes; Concurrent no treatment
NOAEL: 50 mg/kg/day
LOAEL: 150 mg/kg/day
Results: In a 24 month chronic toxicity/carcinogenic study, the test substance was administered orally, via dietary admixture, to groups of six-week old CD male and female rats (75/sex/group). Diets were prepared weekly and samples were analyzed for test compound content. Control animals received the standard laboratory diet. Animals were observed daily for mortality and signs of toxicity. Body weights were recorded weekly for the first 14 weeks, every two weeks from weeks 16-30, and every four weeks after week 30. Feed consumption was measured for 15 rats/sex/dose group during these same periods. Five males and five females in each dose group were sacrificed at 6 months; 10 males and 10 females were sacrificed at 12 and 18 months. Blood was collected from all animals sacrificed at 6, 12 and 18 months, and at termination of the study. At the termination of the study, 10 animals/sex/dose were examined for clinical chemistry and haematology values. Serum samples were prepared for clinical chemistry evaluation (glucose, urea nitrogen, glutamic oxaloacetic transaminase, alkaline phosphatase, gamma-glutamyl transpeptidase, glutamic pyruvic transaminase). Animals sacrificed at six months did not have SGPT levels measured. Hematological parameters (hematocrit, haemoglobin, erythrocyte, leukocyte, platelet and reticulocyte counts) were determined at the same interval as above, using different animals from those used for the clinical chemistry assay. Urine analysis parameters (appearance, specific gravity, pH, protein, glucose, blood cells) were also determined at the same intervals for the rats used for haematology determinations. All animals in the study were necropsied and examined for gross pathological lesions following their natural death or sacrifice. Organ weights and organ/body weight ratios were recorded for brain, pituitary, heart, liver,

adrenals, kidneys, spleen and gonads. Histopathologic examinations were conducted on tissues and organs from all animals in the study. Tissues examined were adrenals, aorta, urinary bladder, bone, bone marrow, cerebellum, cerebrum, cecum, colon, esophagus, eye and optic nerve, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, lymph nodes, mammary glands, skeletal muscle, sciatic nerve, ovaries, pancreas, parathyriods, penis, pituitary, prostate, salivary glands, seminal vesicles, skin, spinal cord, spleen, stomach, testes, thymus, thyroids, trachea, uterus, vagina, and any other tissues with gross lesions. Histopathologic evaluations were conducted only on control and high-dose animals from the six-month sacrifice. Statistical evaluations included mean body weight, mean food consumption, mean clinical laboratory values, and mean terminal organ/body weight and organ/body weight ratios via the appropriate one-way analysis of variance technique, followed by a multiple comparison procedure. Calculations for the statistical significance of differences were performed according to the method of Dunnett (1955). Rat feed was analyzed throughout the study via HPLC analysis to confirm dose levels, homogeneity and stability of the dietary admixture.

Chronic Toxicity Findings: Survival of male rats was somewhat lower in treated versus control groups. At the end of 23 months on study, survival of the high-dose males was 13/50 animals, excluding the interim sacrifices. Based on this lower survival rate, males were terminated at this time. Females were terminated at the end of 24 months, at which time survival was comparable in all dose groups (16-18 animals/group). Clinical signs observed included alopecia, skin lesions, nasal discharge, diarrhea and footpad granulomas. However, there was no distribution of these signs that indicated a relationship to treatment with the test compound. Mean weekly body weights for males in the control and low-dose groups were similar throughout the study. Males in the mid-dose group had significantly lower body weights throughout most of the study. Males in the high-dose group showed significantly lower body weights throughout the entire study. For females, the body weight for low- and mid-dose groups were lower than, but parallel to, controls throughout the study, with occasional statistical differences. By the end of the study, there was no clear statistical difference between these groups. Body weights for high-dose females were significantly different from controls throughout the study, with the exception of the final few weeks. Food consumption for both males and females at all treatment levels was similar to that of the controls. No consistent changes in clinical chemistry parameters were observed in male and female rats, though several significant differences were noted in high-dose animals at various observation periods. Values for GGTP were significantly different for high-dose females at 12, 18 and 24 months, and for mid-dose females at 24 months. No determination could be made on glucose, SGOT or SGPT values for males at 23 months due to hemolysis of blood samples. In the haematological determinations, males exhibited decreases in erythrocytes at the high-dose level. High-dose males and females exhibited decreased haemoglobin and hematocrits throughout the

study. The incidence for rats exhibiting abnormal protein values in the urine was increased compared to historical values for this species and strain. This increased incidence was most prominent in males, and substantially less so in females. The high protein values were considered to be related to the presence of glomerulonephrosis, a common lesion observed in this species and strain of rats. Changes in absolute organ weights or in organ-to-body-weight ratios were considered, for most tissues, to be either random occurrences or a reflection of the decreased body weights that occurred later in the study, rather than a sign of organ toxicity. Liver weights and liver-to-body-weight ratios were elevated for males in all treatment groups. For high-dose males, these differences persisted throughout the study; mid- and low-dose males showed increases only at the end of the study. Females had increased liver weights at all treatment levels. Increased liver-to-body-weight ratios were present for high-dose females at all sacrifice periods. Males demonstrated increased kidney weights throughout the study. At the time of necropsy, gross pathologic examination of animals at post-mortem revealed no lesions that were clearly attributed to treatment. Liver discoloration was present in both males and females, and mammary nodules were present in females. In the histopathologic evaluation of these tissues, the most common findings attributable to treatment were fatty infiltration of the liver and bile duct hyperplasia present in in high-dose males and mid- and high-dose females. A higher incidence of these findings was reported for females exhibiting liver necrosis.

Tumor/Carcinogenic Findings: See Section 5.7, Carcinogenicity

Method: Other: PRL Protocol, 1977, approved by study sponsor; IARC Long Term and Short Term Screening for Carcinogens

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 97.2%

Reference: Monsanto PR-78-109, Pharmacopathics Research Laboratories, 1984

Reliability: (1) Valid without restriction

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type: Ames Bacterial Reverse Mutation Assay

System of testing: Salmonella typhimurium TA-1535, TA-1537, TA-1538, TA-98, TA-100

Concentration: 0.1, 1.0, 10, 100 and 500 micrograms/plate

Metabolic activation: With and without

Results:

Cytotoxicity conc: With metabolic activation: 500 ug/plate
Without metabolic activation: 500 ug/plate

Precipitation conc: None

Genotoxic effects:

With metabolic activation: Negative
Without metabolic activation: Negative

Method: Ames Mutagenicity Plate Assay (Overlay Method), 1975

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: >96%

Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 10 ug/plate methylnitrosoguanidine (MNNG), 100 ug/plate 2-nitrofluorene (NF) and 10 ug/plate quinacrine mustard (QM). Positive control chemicals used for the activation assays were 100 ug/plate 2-anthramine (ANTH), 100 ug/plate 2-acetylaminofluorine (AAF) and 100 ug/plate 8-aminoquinoline (AMQ). 2.5% Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-76-223, Litton Bionetics, 1976

Reliability: (1) Valid without restriction

B. NON-BACTERIAL IN VITRO TEST

Type: Mitotic Recombination Assay

System of testing: Saccharomyces cerevisiae Strain D4

Concentration: 0.1, 1.0, 10, 100 and 500 micrograms/plate

Metabolic activation: With and without

Results:

Cytotoxicity conc: With metabolic activation: 500 ug/plate
Without metabolic activation: 500 ug/plate

Precipitation conc: None

Genotoxic effects:

With metabolic activation: Negative
Without metabolic activation: Negative

Method: Ames Mutagenicity Plate Assay (Overlay Method), 1975

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: >96%

Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. The chemical used as the positive control for the non-activation assay was methylnitrosoguanidine (MNNG) at 10 ug/plate. Positive

control chemical used for the activation assay was DMNA at 100 micromoles/plate. Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-76-223, Litton Bionetics, 1976

Reliability: (1) Valid without restriction

Type: Mouse Lymphoma Forward Mutation Assay

System of testing: L5178Y

Concentration: 0.5, 1.0, 2.0, 4.0 and 8.0 ug/ml with activation
0.125, 0.250, 0.50, 1.0, 2.0 and 4.0 ug/ml without activation

Metabolic activation: With and without

Results:

Cytotoxicity conc: With metabolic activation: 8.0 ug/ml
Without metabolic activation: 4.0 ug/ml

Precipitation conc: Not determined

Genotoxic effects:

With metabolic activation: Negative
Without metabolic activation: Negative

Method: Other: Clive, D. and Spector, J.F.S. (1975)
Mutation Res. 31, 17-29

GLP: No data

Test substance: As prescribed in 1.1-1.4, purity: 98+%

Remarks: The test substance was dissolved in DMSO at 250 ug/ml. Working solutions were made of this stock solution by making a series of two-fold serial dilutions with DMSO. One tenth ml of each stock solution or one of the working dilutions was added to 3x10(6) cells in 10 ml of medium to achieve the desired final concentration. DMSO (1%) was used as the solvent control substance. Growth medium without the addition of solvent was used as a negative control. No genetic effects were attributed to the presence of the solvent. EMS and DMN were used as reference mutagens and induced mutation frequencies within the expected range. Concentrations of 4.0 ug/ml proved to be highly cytotoxic in the absence of an activation system, but less toxic in the presence of an activation system.

	Conc.	Mutant clones	Viable clones	Mutant frequency x10E-4
Non-Activation				
Solvent Control	---	126.0	200.0	0.6300
Negative Control	---	108.0	147.0	0.7347
EMS	0.2 ul/ml	168.0	148.0	1.1351
Test Compound	0.125 ug/ml	54.0	174.0	0.3103
	0.250 ug/ml	120.0	211.0	0.5687
	0.500 ug/ml	85.0	199.0	0.4271
	1.000 ug/ml	108.0	186.0	0.5806
	2.000 ug/ml	117.0	220.0	0.5381
	4.000 ug/ml	TOXIC		

Activation with S-9

Solvent Control	---	124.0	165.0	0.7515
DMN	0.3 ul/ml	85.0	28.0	3.0357
Test Compound	0.500 ug/ml	100.0	191.0	0.5236
	1.000 ug/ml	103.0	166.0	0.6205
	2.000 ug/ml	87.0	147.0	0.5918
	4.000 ug/ml	97.0	164.0	0.5915
	8.000 ug/ml	80.0	164.0	0.4748

The test substance was considered to be not active in the L5178Y Mouse Lymphoma Assay.

Reference: Monsanto BO-76-247, Litton Bionetics, 1977

Reliability: (1) Valid without restriction

Type: *In vitro* Unscheduled DNA Synthesis (UDS)

System of testing: Primary rat hepatocyte cultures (Fischer-344 strain)

Concentration: Preliminary: 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0
Replicate: 1.0, 5.0, 10.0, 50.0, 100.0, 200.0

Metabolic activation: With and without

Results:

- Cytotoxicity conc: Preliminary Assay: 50.0 ug/ml
  Replicate Assay: 100 and 200 ug/ml
- Precipitation conc: Not determined
- Genotoxic effects: Negative

Method: Williams, G.M., Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures, Cancer Research 37, pp. 1845-1851 (1977);
OECD 482 equivalent

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 98%

Remarks: Acetone (1%) used as solvent and diluent. Primary rat liver cell cultures derived from the livers of two adult male rats weighing 248 and 284 grams (both 13 weeks old) were used for the preliminary and replicate experiments, respectively. Three controls were incorporated into each UDS assay: a positive control, a negative (solvent) control, and an untreated medium control. The positive control was 2-Acetylaminofluorene (2-AAF), the solvent control was acetone in the preliminary assay and in the replicate assay. The percentage of cells in repair was calculated as the percentage of cells with at least 5 net grains/nucleus. 150 cells were scored for each concentration reported for each experiment. All collection of data and pooling of slides were done via programs in the VAX 11/782 computer. The net grain counts were negative at each concentration of the test compound, in the solvent control and in the medium control, in contrast to the strong positive response produced by the positive control 2-AAF in both experiments (37.7 and 33.2 net grains/nucleus). An unusual discrepancy was observed in the preliminary experiment. Slides from cultures tested at 100 ug/ml were considered scorable, while slides from cultures tested at 50 ug/ml were considered cytotoxic. Replicate testing indicated cytotoxicity at 100 and 200 ug/ml, but not at 50 ug/ml. UDS was measured at concentrations of the test compound between 0.5 and

100 ug/ml in the preliminary assay and between 1.0 and 50 ug/ml in the replicate assay. UDS was not measured at 0.005, 0.01, 0.05 and 0.1 ug/ml because UDS was not observed at these concentration levels. The results indicate that the test compound is not a genotoxic agent under the conditions of the *in vitro* rat hepatocyte DNA repair assay.

Treatment	Conc.	NG	SE	Median	%IR
DMSO	1%	-10.8	2.0	- 9.0	1
2-AAF ug/ml	5.0	33.2	8.5	33.5	90
Test Cpd. ug/ml	1.0	-12.0	3.3	-10.7	2
	5.0	- 9.8	0.6	- 9.2	1
	10.0	-12.9	3.6	-11.6	1
	50.0	-11.7	3.3	- 9.7	7
	100.0	TOXIC			
	200.0	TOXIC			

Reference: Monsanto SR-83-283, SRI International, 1984
Reliability: (1) Valid without restriction

Type: CHO/HGPRT Forward Gene Mutation Assay
System of testing: CHO Cells, clone K1-BH4
Concentration: Cytotoxicity: 0.33, 1.0, 3.33, 10, 33.3, 100, 333 and 1000 ug/ml
Preliminary Mutation: 1-100 ug/ml
Mutation: 1-30 ug/ml
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 100 ug/ml with 2% S-9
Without metabolic activation: 33.3 ug/ml with 2% S-9
Precipitation conc: Not determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: CHO/HGPRT Mutation Assay (1979) Hsie, et.al.
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 98%
Results: Stock cultures of the CHO-K1-BH4 line were maintained in frozen aliquots. Fresh cultures of the cell line were prepared from frozen stock cultures known to have a stable spontaneous mutation frequency of 0-10 x 10 (-6) mutants/cell.
In order to validate the integrity of the test system, known positive chemicals were run concurrently. Positive control chemicals used were EMS (ethylmethanesulfonate, 200 ug/l) and DMN (dimethylnitrosamine, 100 ug/ml). EMS is a direct acting mutagen, while DMN requires metabolic activation.
The activation system was Aroclor 1254 induced rat liver homogenate (S-9 fraction).
DMSO was used as the solvent and solvent control.
All statistical analysis was performed using the one-way analysis of variance method outlined by Snee and Irr (1981) as well as the one-tailed student's t-test.

Cytotoxicity Experiment

The test compound was evaluated for cytotoxicity in CHO cells at dose levels of 0.33, 1.0, 3.33, 10, 33.3, 100, 333 and 1000 ug/ml of treatment volume at concentrations of 1, 2, 5 and 10% metabolic activation (S-9) preparation and without metabolic activation (S-9). The results produced a total cytotoxic effect at the 333 and 1000 ug/ml levels at all concentrations with and without S-9 activation. The results at the 100 ug/ml level produced a 0%, 0%, 29% and 64% relative cell survival at the 1%, 2%, 5% and 10% concentrations of S-9 activation, respectively, and a 1% relative survival without S-9. The results at the 33.3 ug/ml level produced a 55%, 74%, 97% and 87% respective cell survival at the 1%, 2%, 5% and 10% concentrations of S-9 activation, respectively, and 12% survival without S-9.

Preliminary Mutagenicity Screening Experiment

The test compound was evaluated in duplicate at dose levels of 1, 3 and 5 ug/ml of treatment volume at the 0% concentration of metabolic activation (S-9) preparation; at dose levels of 1, 3 and 10 ug/ml at the 1% S-9 concentration; at dose levels of 3, 10 and 30 ug/ml at the 2% S-9 concentration; at dose levels of 10, 30 and 60 ug/ml at the 5% S-9 concentration; and at doses of 10, 30 and 100 ug/ml at the 10% S-9 concentration. The results of this assay produced no significant increases in the mutation frequencies observed in the treated cultures (see table below). After critical evaluation, a choice was made to use the 2% metabolic activation preparation in the final mutagenicity assay based upon the similar response in mutation frequency at 10 and 30 ug/ml obtained in the Preliminary Mutagenicity Assay.

Preliminary Experiment

S9	Test Compound	Mean Mutant Frequency
0%	Solvent Control	11.3
	1 ug/ml test cpd.	18.4
	3 ug/ml	12.6
	5 ug/ml	13.2
	EMS	215.3
1%	Solvent Control	9.3
	1 ug/ml test cpd.	22.7
	3 ug/ml test cpd.	14.6
	10 ug/ml test cpd.	30.0
2%	Solvent Control	9.4
	3 ug/ml test cpd.	9.3
	10 ug/ml	19.6
	30 ug/ml	19.5
	DMN	216.0
5%	Solvent Control	7.2
	10 ug/ml test cpd.	25.7
	30 ug/ml	10.0
	60 ug/ml	15.5

10%	Solvent Control	6.0
	10 ug/ml test cpd.	11.6
	30 ug/ml	25.2
	100 ug/ml	9.6

CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay

The test compound was evaluated in triplicate at dose levels of 1, 5, 7.5, 10 and 12.5 ug/ml of treatment volume without (0%) metabolic activation, and at doses of 1, 3, 10, 25 and 30 ug/ml of treatment volume with a 2% (v/v) concentration of the metabolic activation S-9 mix. The results of this assay produced no statistically significant increase in the mutation frequencies of cultures treated with the test compound.

Confirmatory Experiment

S9	Test Compound	Mean Mutant Frequency
0%	Solvent Control	9.3
	1 ug/ml test cpd.	22.5
	5 ug/ml	16.5
	7.5 ug/ml	19.0
	10 ug/ml	23.3
	12.5 ug/ml	19.2
	EMS	342.3
2%	Solvent Control	7.9
	1 ug/ml	9.8
	3 ug/ml	4.2
	10 ug/ml	5.4
	25 ug/ml	6.3
	30 ug/ml	9.0
	DMN	220.7

Reference: Monsanto PK-83-279, Pharmakon Research, 1984
Reliability: (1) Valid without restriction

* 5.6 GENETIC TOXICITY IN VIVO

Type: *In vivo* Unscheduled DNA Synthesis (UDS)/S-Phase
Species/strain: Rats, Sprague-Dawley
System of testing: Primary rat hepatocyte cultures
Sex: Female
# of animals: 32
Route of Administration: Oral gavage
Vehicle: Corn Oil
Exposure period: 2, 16 or 48 hours before sacrifice
Frequency of treatment: Single dose
Dose: 0, 100, 500 and 1000 mg/kg bw
Control Group: Yes, concurrent vehicle and positive controls
Results:
Genotoxic effects: Negative
Method: Mirsalis, J.C., *In vivo* measurement of unscheduled DNA systhesis and hepatic cell proliferation as an indicator of hepatocarcinogenesis in rodents. J. Appl. Toxicol. 1986;

OECD 486 equivalent

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >95%

Remarks: Female Sprague-Dawley rats were exposed to the test compound by gavage. The test compound was suspended in corn oil. A volume of 1.0 ml of this solution was administered per 100 g body weight of the test animals. Animals used for examination of UDS were sacrificed at either 2 or 26 hours post-treatment. Animals used for S-phase examination were sacrificed at 48 hours post-treatment. At least four control groups (two positive and two negative) were used in each assay. The positive controls were DMN (dimethylnitrosamine, 10 mg/kg in water) for UDS, and MP (methaprylene, 225 mg/kg in corn oil) for S-phase; each corresponded to the time point and route of administration of the test compound. Both DMN and MP are known hepatocarcinogens requiring metabolic activation and produce positive responses in these assays. An additional DMN group was added at the 2-hr exposure time, for a total of four DMN-treated animals. Primary liver cell cultures were obtained from the livers of treated rats according to the procedure of Mirsalis et. al (1982) and inoculated into culture dishes and supplemented with l-glutamine, gentamicin sulphate and 10% fetal bovine serum. Following incubation, cell cultures were washed, swelled, fixed, and then washed again prior to staining with 1% methyl-green Pyronin Y. Measurement of UDS: Quantative audiographic grain counting was done using colony counters interfaced with a microscope via a TV camera. Data were fed directly into a VAX 8800 computer. Thirty morphologically unaltered cells on a randomly selected area of each slide were counted. The highest count from two nuclear-size areas over the most heavily labelled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains/nucleus (NG). The percentage of cells in repair, indicative of the extent of the response throughout the liver, was calculated as those cells exhibiting at least 5 NG. All collection of data and pooling of slides or animals were done by programs in the VAX 8800 computer. A minimum of three slides were scored for each of three animals, for a minimum total sample of 3 animals, 9 slides and 270 cells/dose/time point. The data generated were considered acceptable if controls were within historical ranges. A test compound was considered "Positive" if any dose group yielded greater than 5.0 NG. A test compound was considered "Negative" if all doses yielded less than 0.0 NG. Values between were considered to be "Equivocal". At all doses and time points, the test compound failed to induce an increase in UDS. The animal-to-animal variation in results was very low for all treatment groups, indicating a high degree of reproducibility.

Treatment	Dose(mg/kg)	Time	# animals	N.G.	% in Repair
Corn Oil	----	16 hr	3	- 3.9	0
DMN	10	2 hr	2	23.1	89 +/- 1
	10	16 hr	2	10.5	56 +/- 30
Test cpd.	100	16 hr	3	- 4.1	0
	500	16 hr	3	- 3.3	0
	1000	16 hr	4	- 3.4	1 +/- 1

S-Phase Study

Data were scored manually for S-phase and pooling of slides or animals was done by programs in the VAX 8800 computer. A minimum of three slides was scored for each of three animals. The total sample included 3 animals, 9 slides and at least 9000 cells/dose/time point. The data generated were considered acceptable if controls were within historical ranges. A test compound was considered "Positive" if the level of DNA replication was markedly elevated above that in the vehicle control. A test compound was considered "Negative" if DNA replication was not markedly elevated above that in the vehicle control. Responses of less than 1.0% S-phase are generally considered to be "Negative" or "Equivocal". No animals receiving any dose of the test compound exhibited an increase in percent of S-phase above that of the negative control, indicating that the test compound does not induce S-phase in female Sprague-Dawley rat liver.

Treatment	Dose(mg/kg)	Time	# animals	% in S-phase
Corn Oil	-----	48 hr	3	0.5 +/- 0.1
MP	225	48 hr	3	1.5 +/- 0.2
Test cpd.	100	48 hr	3	0.4 +/- 0.2
	500	48 hr	3	0.2 +/- 0.1
	1000	48 hr	3	0.5 +/- 0.4

Reference: Monsanto SR-86-242, SRI International, 1986
Reliability: (1) Valid without restriction

Type: *In vivo* Mammalian Bone Marrow Chromosomal Aberration Test
Species/strain: Rats, Charles River Sprague-Dawley CD
Sex: Male and Female
# of animals: 70
Route of Administration: Oral gavage
Vehicle: Corn oil
Exposure period: 6, 24 and 48 Hours
Doses: 0 and 3000 mg/kg
Results:
Effect on mitotic index or P/N ratio: No effect
Genotoxic effects: Negative
Method: In vivo Bone Marrow Cytogenetics Rat Metaphase Analysis 1981 OECD 475 Equivalent
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 95%
Remarks: A single dose of the test compound was administered by oral gavage to two groups of 15 male and 15 female rats at levels of 0 and 3000 mg/kg body weight. A positive control chemical, Cyclophosphamide (40 mg/kg) was also administeed to 5 male and 5 female animals. Observations of general appearance, behaviour, toxic and pharmacological effects were recorded twice daily and prior to sacrifice. Body weights were recorded once, prior to compound administration, for the 6-hour sacrifice group,

and twice (prior to compound administration and prior to colchicine administration) for the 24- and 48-hour sacrifices. At approximately 4, 22 and 46 hours after administration of the test and control substances, the animals received a single intraperitoneal injection of colchicine (2.0 mg/kg bw, dosing factor 5 ml/kg) to inhibit mitosis and arrest cells in metaphase. Two hours after colchicines, the animals were sacrificed via CO2 asphyxiation. Bone marrow cells were processed according to the modified techniques described by Evans (1976) and Killian et. al. (1977). After all slides were prepared, stained and coverslipped, a code number was assigned to each animal by a person not involved in the scoring of the slides. The slides were not decoded until all had been analyzed. Fifty cells (if possible) were examined from each rat that provided analyzable cells. Upon completion of all scoring, slides were decoded and the data entered into the appropriate group for statistical analysis. The mean mitotic indices, mean chromosome numbers, % aberrant cells, and the mean number of aberrations per cell for each group were statistically compared using the Kruskal-Wallis nonparametric analysis of variance and nonparametric pairwise group comparisons (KW-ANOVA). Body weight data was analyzed by analysis of covariance (ANCOVA). All tests were one-tailed at the 95% confidence interval ($P<0.5$). Clinical signs of toxicity were evident – wheezing, depression, red stains on nose and eyes, urine stains, labored respiration, and one male and four females died before sacrifice. A significant variance in mean body weight changes was seen in both sexes of the 3000 mg/kg animals at 24 hours, and in males at 48 hours. These pharmacotoxic signs indicated that the test article was administered at or near the maximum tolerated dose. No statistically significant increase in the frequency of chromosomal aberrations compared to control values was seen in the animals treated with the test compound. A statistically significant increase in % aberrant cells ($p=0.0155$) and the average number of aberrations per cell ($p=0.0155$) was seen in the animals treated with the positive control chemical Cyclophosphamide. No statistically significant differences between the mean chromosome numbers of the test group and the vehicle control group were seen. The number of cells undergoing mitosis per 500 cells counted (mitotic index) was determined for each animal. No statistically significant difference between the mean mitotic indices of the test group and the vehicle control were seen. Therefore, under the conditions of this study, the test compound was not considered to be clastogenic.

<u>6 Hour Sacrifice</u>

Treatment	Mean chromosome #	Mean MI	% aberrant cells
Corn Oil	41.98	2.64	0
Test Cpd.	41.92	2.64	0

<u>24 Hour Sacrifice</u>

Treatment	Mean chromosome #	Mean MI	% aberrant cells
Corn Oil	41.78	2.80	0
Positive Control	41.82	1.94	3.36
Test Cpd.	41.80	2.64	0

48 Hour Sacrifice

Treatment	Mean chromosome #	Mean MI	% aberrant cells
Corn Oil	41.86	1.80	0
Test Cpd.	41.86	1.52	0

Reference: Monsanto HL-84-160, Hazleton Laboratories, 1985
Reliability: (1) Valid without restriction

*5.7 CARCINOGENICITY

Species/strain: Rats, Sprague-Dawley CD
Sex: Male/Female
# of animals: 600 (75/sex/dose)
Route of Administration: Dietary
Exposure period: 23 months for male rats
24 months for female rats
Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 50, 150 or 500 mg/kg/day
Control group: Yes; Concurrent no treatment
NOAEL: 500 mg/kg/day for males
500 mg/kg/day for females for malignant tumors
50 mg/kg/day for females for benign tumors
LOAEL: Not determined for males
150 mg/kg/day for benign tumors in females
Results: Study details: See Section 5.4, Repeat Dose
Chronic Toxicity Findings: See Section 5.4, Repeat Dose
Carcinogenic Findings: There were no significant differences for treated animals of either sex in the number of total tumors, in the number of benign or malignant tumors, or in the number of animals which had tumors. There was no significant difference between control and high-dose animals for any individual tumor type, with the exception of benign liver adenomas. Female mid- and high-dose animals exhibited a significant increase in benign liver adenomas both in animals that died on study and in those that survived until the end of the study. The total incidence for this tumor was four adenomas in the mid-dose females, and eleven adenomas in the high-dose females. For males, liver adenomas were seen only in one low-dose and one mid-dose animal. An analysis of tumors present in animals which died on study indicated no differences between control and treated animals in the number of animals whose death could be attributed to the presence of a tumor. Due to the lack of progression progression from benign to malignant lesions, the lack of proliferative lesions in males or at other sites, and negative results in genetic toxicology studies, the benign liver tumors were not considered to pose a significant human health risk.

Liver Tumor Incidence Summary

Adenoma	Dose 0 mg/kg/day	Males 0	Females 0
	50 mg/kg/day	Males 1	Females 0
	150 mg/kg/day	Males 1	Females 4
	500 mg/kg/day	Males 0	Females 11

Method:	Other: PRL Protocol, 1977, approved by study sponsor; IARC Long Term and Short Term Screening for Carcinogens
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 97.2%
Reference:	Monsanto PR-78-109, Pharmacopathics Research Laboratories, 1979
Reliability:	(1) Valid without restriction

***5.8 TOXICITY TO REPRODUCTION**

Type: One-generation
Species/strain: Rats, Long-Evans
Sex: Male/Female
Route of Administration: Dietary
Exposure period: F0 females: gestation through lactation
F1 males/females: growth, mating, gestation and lactation (2 successive litters, F2a and F2b)
Frequency of treatment: Daily
Post exposure observation period: None
Premating exposure period: F0 male: None, F0 female: None
F1 male: Continuous, F1 female: Continuous
Duration of the test: 288 days
Doses: 0, 50, 150 and 500 ppm
Control group: Yes; Concurrent no treatment
NOEL Parental: 150 ppm for both sexes [based on body weights]
NOEL F1 Offspring: 50 ppm for males [based on body weights, organ/body weights]
150 ppm for females [based on body weights]
NOEL F2 Offspring: 150 ppm [based on 24-hour survival index]
Results: **General parental toxicity:**

F0 females: No mortalities occurred in either the control or treated groups during the gestation or lactation periods. Mean maternal body weights for the high-dose animals were slightly lower than the combined mean control data during most of the gestation and lactation periods. However, mean weight gain data during the gestation and lactation periods were comparable between the control and treated groups. Mean food consumption was comparable in all groups. The type and incidence of physical observations were comparable in all groups during gestation and lactation. Pregnancy rates were comparable in all groups. Necropsy observations indicated no treatment-related effects. All organ weights and ratios for the treated groups were comparable to the control data. Mating and fertility indices (male and female) were comparable between the control and treated groups. Pregnancy rates were comparable between controls and treated groups.

F1 females: One control group female died spontaneously on Day 38 of the growth period. One control group female died spontaneously on Day 18 of the F2a lactation period. There were no mortalities in treated females. The mean body weights of the high-dose females were slightly lower than the pooled controls during the growth period and for part of the rest period. Mean body weight gains for the females during the growth period were considered comparable between the pooled controls, low- and

mid-dose groups, and slightly lower than control at the high-dose level. Mean food consumption was comparable in all groups. The type and incidence of physical observations were comparable in all groups during gestation and lactation. Pregnancy rates were comparable in all groups. Necropsy observations indicated no treatment-related effects. All organ weights and ratios for the treated groups were comparable to the control data. Mating and fertility indices (male and female) were comparable between the control and treated groups. Pregnancy rates were comparable between controls and treated groups.

F1 males: One low-dose male died spontaneously on Day 26 of the growth period; there were no other mortalities. The mean body weights of the high-dose males were slightly lower than the data for the pooled controls during the growth and rest periods. Mean food consumption was comparable in all groups. The type and incidence of physical observations were comparable in all groups. Necropsy observations indicated no treatment-related effects. Mean kidney weights in the high-dose males were significantly higher than the pooled control value, and relative kidney weights (to body weights and brain weights) were significantly higher than the pooled control value in both the mid- and high-dose animals. All other organ weights and ratios were considered comparable to control values. Mating and fertility indices (male and female) were comparable between the control and treated groups.

Toxicity to offspring:

F1 generation: Mean gestation length was comparable between the control and treated groups. Pup survival indices for the control and treated groups were comparable at birth as well as on Days 1, 4, 14 and 21 of lactation. Mean pup body weights of the control and treated groups were comparable at birth and on Days 4, 14 and 21 of lactation. No treatment-related effects were seen in pup sex distribution data. Necropsy observations of the F1 pups not selected as parents for the next generation indicated no treatment-related effects. The results of gross post-mortem and microscopic examination of tissues from ten high-dose F1 parents (5/sex) revealed no morphologic change related to ingestion of the test compound.

F2a generation: Mean gestation length was comparable between the control and treated groups. The mean numbers of live and dead pups at birth were comparable in the control, low- and mid-dose groups. In the high-dose group, the mean number of live pups at birth was significantly reduced, and the mean number of dead pups was increased. The difference in the high-dose group was mainly attributed to one female which lost an entire litter of eleven pups. Mean pup body weights and sex distribution ratios were comparable to controls in all treated groups. No treatment-related effects were noted in pup necropsy examinations.

F2b generation: Mean gestation length was comparable between the control and treated groups. The mean numbers of live pups at birth were slightly reduced in all treated groups. However, the mean number of dead pups was comparable in treated and control animals. The percentage of females that weaned litters was comparable between the control and treated groups for both the

first and second lactation intervals. The 24-hour survival index for the high-dose group was significantly lower than the values for controls. However, the 4-, 14- and 21-day pup survival indices for high-dose animals were comparable to controls. High-dose pup survival indices were also comparable to controls throughout lactation. Mean pup body weights and sex distribution ratios were comparable to controls in all treated groups. No treatment-related effects were noted in pup necropsy examinations.

Method: Administration of the test substance to the F0 females was begun on the day signs of mating were observed (Day 0 of gestation) and continued throughout the ensuing gestation and lactation periods. F1 offspring were separated from siblings seven days after the weaning (Day 21 of lactation) of the last litter and randomly selected to continue as future parents (F1). More offspring than needed were selected (12 males and 24 females) for the growth period to ensure the required number of adults (10 males and 20 females) necessary for mating. Following pup selection, the remaining offspring and F0 females were sacrificed. Gross external and internal exams were performed on all sacrificed animals. The parentage of each selected offspring was recorded to avoid possible brother-sister mating. F1 rats were raised to maturity and mated to produce the F2a litters. F2a pups were sacrificed and necropsied at weaning. All F1 females were remated after a rest period of at least 14 days to produce the F2b litters. F2B pups were sacrificed and necropsied at weaning. Following completion of the F2b sacrifice, all F1 parents were sacrificed, necropsied, and selected tissues were preserved. Diet samples were prepared fresh weekly and analyzed weekly via HPLC to guarantee the accuracy of the doses levels. Body weights were recorded weekly during growth and rest periods. Body weights of pregnant females (F0, F1) were recorded on Days 0, 6, 15 and 20 of gestation. Body weights of lactating females (F0, F1) were recorded on Days 0, 4, 14 and 21 of lactation. Food consumption was recorded weekly during the growth and rest periods of the F1 generation. F0 and F1 parents were observed twice daily for mortality and gross signs of toxicologic or pharmacologic effects. Detailed physical examinations were conducted weekly on the F0 and F1 parents. Pups (F1, F2a, F2b) were examined daily for mortality and general appearance. Pups were counted on Days 0, 1, 4, 14 and 21 of lactation. Live pups were weighed as a litter (F1, F2a, F2b) on Days 0, 4, 14 and 21 of lactation, and individually on Day 21 of lactation. External sex determination of pups (number of each sex per litter, F1, F2a, F2b) was recorded on Days 0, 4 and 21 of lactation, and individually on Day 21 of lactation. Body weight, body weight gain, food consumption and litter examination data, organ weights, organ/body weight and organ/brain weight ratios were analyzed and compared to controls using the methods of Dunnett (1964), the F-test and Student's t-test with Cochran's approximation (1967), and the applicable chi-square method of analysis. Pregnancy rates and sex distribution ratios were calculated for all groups. The following live birth and survival indices were calculated: Live Birth Index, 24-Hour Survival

Index, 4-Day Survival Index, 14-Day Survival Index, 21-Day Survival Index.

GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99+%
Remarks: No consistent reproductive effects were noted in this study.
Reference: Monsanto BD-77-356, Bio/Dynamics, Inc., 1979
Reliability: (1) Valid without restriction

*5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

Species/strain: Rabbits, New Zealand White
Sex: Female
# of animals: 72 (18/dose level)
Route of Administration: Gastric intubation
Duration of the test: 24 days
Exposure period: Day 7-19 of gestation
Frequency of treatment: Daily
Doses: 0, 10, 30 or 100 mg/kg/day
Vehicle: Corn Oil
Control group: Yes; Concurrent vehicle
NOEL Maternal Toxicity: 30 mg/kg/day [based on weight loss]
NOEL teratogenicity : 100 mg/kg/day
Results: The test compound was administered to pregnant New Zealand White rabbits via gastric intubation from Day 7 through Day 19 of gestation. The test animals were 5-6 month old virgin females at time of mating and weighed 2.98 to 4.20 kg. Test animals were observed twice daily for signs of toxicologic or pharmacologic effects. Detailed physical exams, including the recording of body weights, were performed on Day 0, 7, 10, 14, 19, 25 and 30 of gestation. The test animals were sacrificed on Day 30 of gestation and uterine implantation data were evaluated. Foetuses recovered at this time were weighed and evaluated for external malformations. All foetuses were then subjected to skeletal as well as soft tissue evaluations. Complete gross post-mortem examinations were performed on all mated females. External surface, all orifices, the cranial cavity, carcass, the external surface of the spinal cord and sectioned surfaces of the brain, nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities, and their viscera and cervical tissues and organs were examined in all animals.

Maternal general toxicity: No mortalities occurred in the control or treated groups. A slight increase in the incidence of alopecia was noted at the 30 and 100 mg/kg/day animals on Days 19 and 25 of gestation. This increase in incidence was slight, and not considered treatment-related. Mean body weights and weight gains were comparable between the control, low- and mid-dose rabbits throughout gestation. At the 100 mg/kg/day dose level, animals exhibited a mean weight loss during the Day 7-19 treatment period that was attributed to treatment. There were no treatment-related adverse effects evident from maternal gross post-mortem evaluations. Statistical evaluation of equality of means was made by the appropriate one-way analysis of variance technique, followed by a multiple comparison procedure if needed. Bartlett's test was performed to determine of groups had

equal variance. If variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one-way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated, Dunn's summed rank test was used to determine which treatments differed from control.

Pregnancy/litter data: Pregnancy rates were 94.4% (Control), 100.0% (10 mg/kg/day), 88.9% (30 mg/kg/day) and 94.4% (100 mg/kg/day). One mid-dose female aborted, and one high-dose female delivered prematurely. No adverse effects of treatment were evident in uterine implantation data.

Foetal data: Mean foetal weight data were lower than control in all treatment groups; however, the differences were not statistically significant. No adverse effect of treatment was evident in foetal sex distribution data. The incidence of foetuses with at least one ossification variance was comparable between the control and treated groups. However, the incidence of high-dose foetuses with specific ossification variations [slightly enlarged cranial fontanel, incompletely ossified frontal bones or hyoid, or asymmetric/unossified 5^{th} or 6^{th} sternebrae] was increased, suggestive of a fetotoxic dose response at 100 mg/kg/day. No adverse effect of treatment was evident in the foetal external, soft tissue or skeletal evaluations. During the skeletal evaluation, the incidence of foetuses with skeletal malformations was comparable between the control group and each of the treated groups. However, in the high-dose group, dissimilar rib/vertebral defects were seen at a slightly higher incidence. In the absence of similarity in the types of vertebral/rib malformations seen at the high-dose level and since only a single foetus within a litter was affected, this slight increase was not considered to be treatment-related.

Method:	USEPA TSCA Guidelines for Teratogenicity Study, Federal Register Vol. 44, No. 145, July 26, 1979
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >97%
Remarks:	Administration of the test substance by gastric intubation to pregnant New Zealand White rabbits at dose levels of 10 and 30 mg/kg/day during Day 7-19 of gestation was not considered maternally toxic, embryotoxic, foetotoxic or teratogenic. At 100 mg/kg/day, maternal toxic effects and foetotoxic effects were seen. The test compound was not considered to be teratogenic at any of the dose levels evaluated.
Reference:	Monsanto BD-83-162, Bio/dynamics Inc., 1985
Reliability:	(1) Valid without restriction

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type of test: Liver Tumor Initiation-Promotion Study
Species/strain: Rats, Sprague-Dawley
Sex: Female
Route of Administration: Dietary
Exposure period: 9 months
Post exposure observation period: None
Frequency of treatment: Daily
Dose: 10,000 ppm
Control group: Yes
Negative control
Initiator control (Diethylnitrosamine)
Promotor control (Phenobarbital)
Positive control (Diethylnitrosamine and Phenobarbital)
Phthalimide (metabolite)
Results: The study was designed to determine the initiation and promotion potential (I-P) of Santogard PVI. Female Sprague-Dawley rats were exposed over a 9 month period to the test compound alone, or in combination with diethylnitrosamine or Phenobarbital in an initiation-promotion protocol. A putative Santogard PVI metabolite (phthalimide, CAS# 85-41-6) was also studies to determine its potential as a promoter. Partial hepatectomy (PH) was used as a mitotic stimulus in I-P animals. There were mortalities during the course of the study in non-hepatectomized animals that were related to exposure to Santogard PVI in the diet (10,000 ppm). Mortality occurred in animals receiving hepatectomies alone and was exacerbated when Santogard PVI was included in the treatment regimen. There were no body weight changes noted except for a weight decrease in those animals receiving partial hepatectomy, diethylnitrosamine and dietary PVI. Food consumption appeared to follow a similar pattern. Gross pathology revealed single or multiple masses/nodules in the livers of this group of animals after six and nine months of treatment. Absolute and relative liver weights were also increased in these animals when compared to controls that only received partial hepatectomies. After nine months of treatment, morphometric analysis was performed on liver specimens for the detection of numbers and volume density of foci and nodules. Santogard PVI appeared to induce promotional activity. Incorporation of systemically administered radiolabeled thymidine into liver specimens of animals following 1 week, 1 month and 6 months of exposure to 10,000 ppm PVI was not increased compared to controls. Dietary exposure to PVI did not appear to induce cell proliferation at these time points. Results of this study indicate that Santogard PVI has promoting activity but has no initiating activity. The reason for the promoting activity of PVI could not be determined. There were no significant increases in enzyme activities associated with peroxisomes, nor could induction of cell proliferation be detected in liver specimens. The metabolite phthalimide did not have promoting activity and apparently does not contribute significantly to the promotional activity of the parent compound.
Reference: Monsanto ML-87-136, Environmental Health Laboratory, 1989
Reliability: (1) Valid without restriction

* 5.11 EXPERIENCE WITH HUMAN EXPOSURE

Results:	Production workers at one PVI manufacturing facility had reported periodic upper respiratory tract irritation during the early years of manufacture.
Remarks:	Enhancements to spot ventilation solved this problem.
Reference:	Monsanto Toxicology Profile of Santogard PVI, 1993

Results:	Complaints of body odor following exposure have been received from production workers at two PVI manufacturing facilities. Workers reported the persistence of odor for several days after cessation of the exposure.
Remarks:	The installation of a sauna eliminated this complaint. Workers will typically use both the shower and sauna after completing their shift.
Reference:	Monsanto Toxicology Profile of Santogard PVI, 1993

6. REFERENCES

1. ASTM D-1519 / Flexsys Physical Methods of Analysis
2. Flexsys Process Manual for Santogard PVI, 1996
3. FF97.8-1, Flexsys Standard Methods of Analysis, 1997
4. EPIWIN/MPBPWIN v1.40, 2000
5. Chemical Hazard Information Profile (CHIP) of CTP, July 24, 1989
6. Monsanto ES-78-SS-20, The Water Solubility and Octanol/Water Partition Coefficient of Selected Rubber Chemicals, Monsanto Environmental Sciences Report, December 13, 1978
7. Monsanto ABC 32456, Santogard PVI Phase I Hydrolysis Study, Analytical Bio-Chemistry Laboratories, February 17, 1986
8. ASTM D 92-96, Test Method for Flash Points and Fire Points by Cleveland Open-Cup Apparatus, Issued 1992, Revised 1996
9. EPIWIN/HENRYWIN v3.10, 2000
10. EPIWIN/AopWin v1.90, 2000
11. Monsanto ABC-32456, Santogard PVI Phase I Hydrolysis Study: Identification of Hydrolysis Products, Analytical Bio-Chemistry Labs, February 17, 1986
12. Monsanto Product Assessment Report 4-2531, Santogard PVI – Safety & Environmental Data, July 20, 1978
13. EPISUITE/EPIWIN 2000
14. EPISUITE/EPIWIN v3.10, 2000
15. Monsanto ES-78-SS-28, Environmental Persistence Screening of Selected Rubber Chemicals, Monsanto Environmental Sciences Laboratories, December 29, 1978
16. Monsanto ES-78-SS-20, The Water Solubility and Octanol/Water Partition Coefficient of Selected Rubber Chemicals, Monsanto Environmental Sciences Laboratory Report, December 13, 1978
17. Monsanto BN-76-252, Acute (96-hour) Toxicity of Santogard PVI to Bluegill, EG&G Bionomics Aquatic Toxicology Laboratory, December, 1976
18. Monsanto BN-76-252, Acute (96-hour) Toxicity of Santogard PVI to Rainbow Trout, EG&G Bionomics Aquatic Toxicology Laboratory, December, 1976
19. Monsanto AB78-122B, Dynamic Toxicity of Santogard PVI to fathead Minnows, Analytical Bio-Chemistry Laboratories, July 30, 1979
20. Monsanto AB-78-122, Acute Toxicity of Santogard PVI to Daphnia magna, Analytical Bio-Chemistry Laboratories, August 25, 1978

21. Monsanto 9AB981016, Acute Toxicity of Santogard PVI to Midge, Analytical Bio-Chemistry Laboratories, September 22, 1981
22. Monsanto BN-78-1384317, Acute Toxicity of Santogard PVI to the Freshwater Alga Selenastrum capricornutum, EG&G Bionomics, September, 1978
23. Monsanto Y-73-259, Acute Oral Toxicological Investigation of Santogard PVI, Younger Laboratories, January 11, 1974
24. Monsanto Y-76-4, Acute Oral Toxicological Investigation of Santogard PVI 25, Younger Laboratories, February 14, 1976
25. Monsanto Y-73-259, Acute Dermal Toxicological Investigation of Santogard PVI, Younger Laboratories, January 11, 1974
26. Monsanto Y-76-4, Acute Dermal Toxicological Investigation of Santogard PVI 25, Younger Laboratories, February 24, 1976
27. Monsanto Y-79-54, Y-79-55, Y-79-56, Y-79-57, Y-79-58, Primary Skin Irritation of Santogard PVI, Younger Laboratories, May 24, 1979
28. Monsanto Y-79-54, Y-79-55, Y-79-56, Y-79-57, Y-79-58, Primary Eye Irritation of Santogard PVI, Younger Laboratories, May 24, 1979
29. Monsanto SH-66-10, Repeated Insult Patch Test Study on Santogard PVI, Industrial Biology Laboratories, 1966
30. Monsanto SH-69-11, Repeated Insult Patch Test Study with Compounded Rubber Stocks Containing Santogard PVI, Industrial Biology Laboratories, January 29, 1970
31. Kanerva, L, Estlander, T., Jolanki, R., Allergic patch test reactions caused by the rubber chemical cyclohexylthiophthalimide, Contact Dermatitis 34, No. 1, p. 23-26, January, 1996
32. Monsanto PR-78-109A, A 4-Week Range-Finding Toxicity Study with Santogard PVI in the Rat via Dietary Admixture, Pharmacopathics Research Laboratories, August 25, 1978
33. Monsanto BTL-76-193, A 4-Week Subchronic Dust Inhalation Study of Santogard PVI in Rats, Industrial Bio-Test Laboratories, June 30, 1977
34. Monsanto BO-84-162, A Thirteen-Week Inhalation Study of Santogard PVI in the Rat, Bio/dynamics, Inc., March 31, 1986
35. Monsanto PR-78-109, Santogard PVI: Chronic Toxicity and Carcinogenic Study in the Rat, Pharmacopathics Research Laboratories, March 10, 1984
36. Monsanto BIO-76-223, Mutagenicity of Santogard PVI, Litton Bionetics, December 30, 1976
37. Monsanto BO-76-247, Mutagenicity Evaluation of Santogard PVI in the Mouse Lymphoma Assay, Litton Bionetics, April, 1977
38. Monsanto SR-83-283, Evaluation of the Potential of Santogard PVI to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures, SRI International, May 21, 1984
39. Monsanto PK-83-279, CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay with Santogard PVI, Pharmakon Research International, Inc., December 04. 1984
40. Monsanto SR-86-242, Examination of Santogard PVI in the *In vivo* Hepatocyte DNA Repair and DNA Replication Assay, SRI International, October 20, 1986
41. Monsanto HL-84-160, *In vivo* Bone Marrow Chromosome Study in Rats with Santogard PVI, Hazleton Biotechnologies Corporation, January 31, 1985
42. Monsanto PR-78-109, Santogard PVI: Chronic Toxicity and Carcinogenic Study in the Rat, Pharmacopathics Research Laboratories, March 10, 1984
43. Monsanto BD-77-356, A One-generation Reproduction Study with Santogard PVI in Rats, Bio/Dynamics, Inc., September 17, 1979
44. Monsanto BD-83-162, A Teratogenicity Study In Rabbits with Santogard PVI, Bio/dynamics Inc., June 6, 1985
45. Monsanto ML-87-136, A Tumor Initiating-Promotion Study with Santogard PVI in Female Rats, Monsanto Environmental Health Laboratory, July 3, 1989
46. Monsanto Toxicology Profile of Santogard PVI, C.E. Healy, February 9, 1993